Figure 1: Novel Gene Sequence Analysis

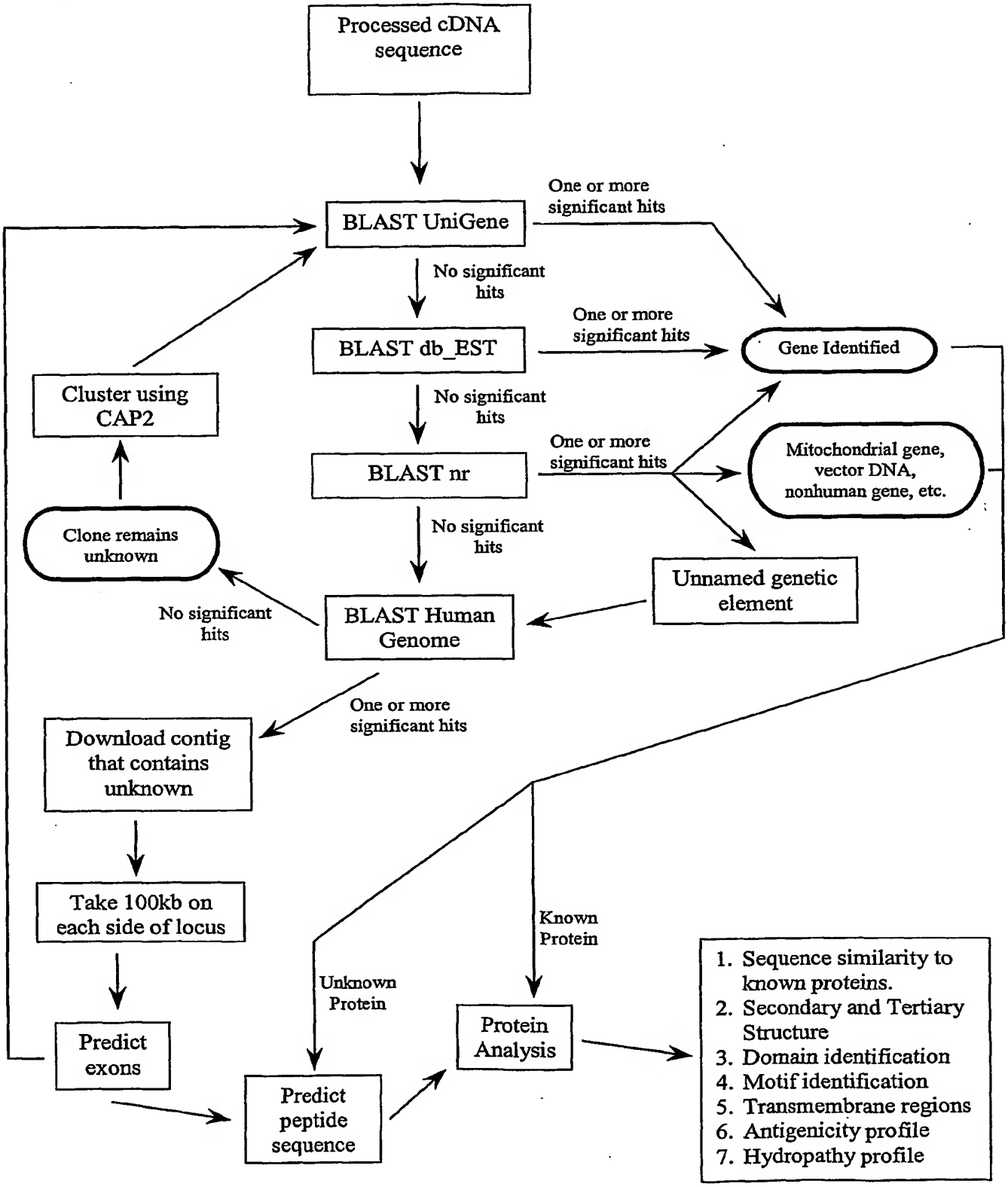


Figure 2: Primer efficiency testing. A standard curve of Ct versus log of the starting RNA amount is shown for 2 genes.

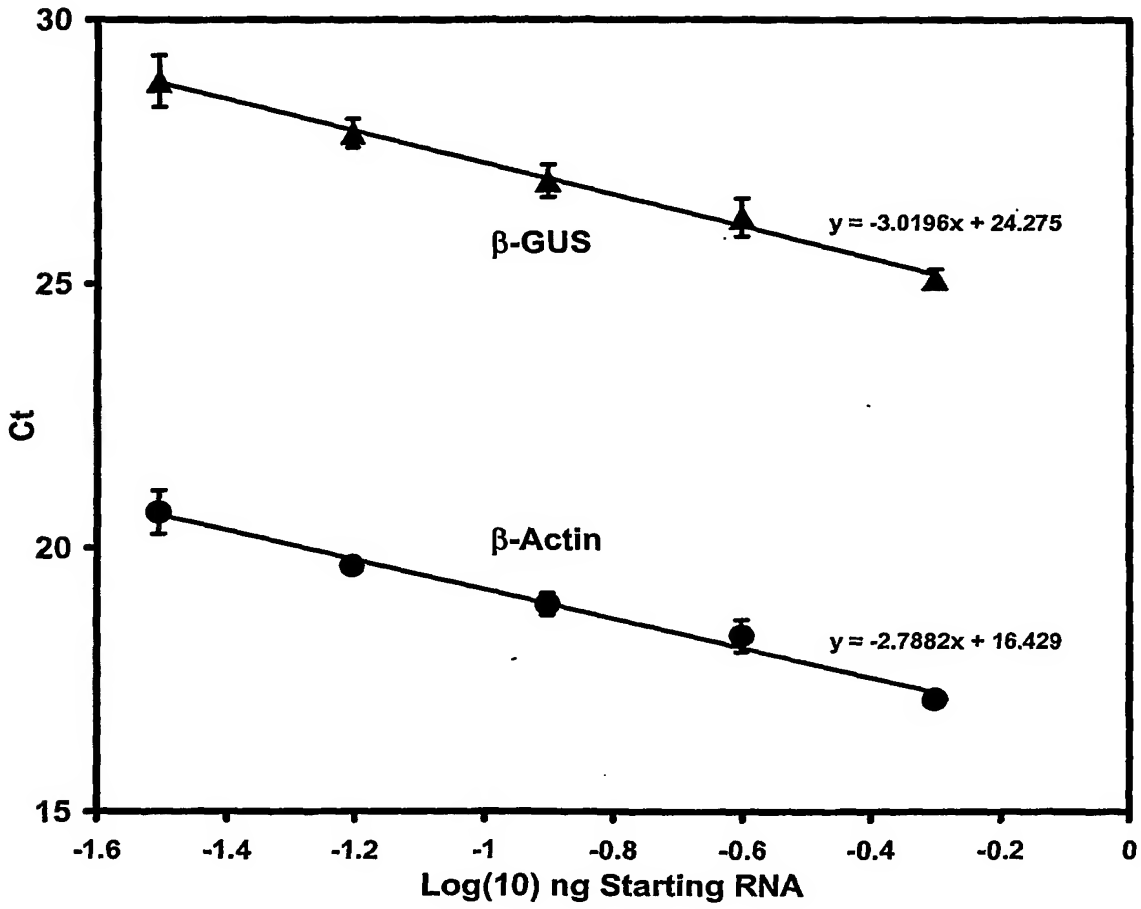


Figure 3: Kits for discovery of, or application of diagnostic gene sets

A. Contents of kit for discovery of diagnostic gene sets using microarrays

1. Sterile, endotoxin and RNAse free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
3.-PBS (phosphate buffer saline; needed when method of example 8 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for labeling of RNA (may vary for various expression profiling techniques)
   - For fluorescence microarray expression profiling:
   - Reverse transcriptase and 10x RT buffer
   - T7(dT)24 primer (primer with T7 promoter at 5' end)
   - DTT
   - Deoxynucleotides 100mM each
   - RNAse inhibitor
   - 2nd strand cDNA buffer
   - DNA polymerase
   - Rnase H
   - T7 RNA polymerase
   - Ribonucleotides
   - In Vitro transcription buffer
   - Cy3 and Cy5 labeled ribonucleotides
7. Microarrays containing candidate gene libraries
8. Cover slips for slides
9. Hybridization chambers
10. Software package for identification of diagnostic gene set from data
    - Contains statistical methods.
    - Allows alteration in desired sensitivity and specificity of gene set.
    - Software facilitates access to and data analysis by centrally located database server.
11. Password and account number to access central database server.
12. Kit User Manual

B. Contents of kit for application of diagnostic gene sets using microarrays

1. Sterile, endotoxin and RNAse free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for labeling of RNA (may vary for various expression profiling techniques)
   - For fluorescence microarray expression profiling:

Reverse transcriptase and 10x RT buffer
T7(dT)24 primer (primer with T7 promoter at 5' end)
DTT
Deoxynucleotides 100mM each
RNAse inhibitor
2nd strand cDNA buffer
DNA polymerase
Rnase H
T7 RNA polymerase
Ribonucleotides
In Vitro transcription buffer
Cy3 and Cy5 labeled ribonucleotides

7. Microarrays containing candidate gene libraries
8. Cover slips for slides
9. Hybridization chambers
10. Software package for identification of diagnostic gene set from data
    Contains statistical methods.
    Allows alteration in desired sensitivity and specificity of gene set.
    Software facilitates access to and data analysis by centrally located database server.
11. Password and account number to access central database server.
12. Kit User Manual

C. Contents of kit for application of diagnostic gene sets using Real-time RT-PCR

1. Sterile, endotoxin and RNAse free blood collection tubes
2. Alcohol swabs, tourniquet, blood collection set
3.-PBS (phosphate buffer saline; needed when method of example 7 is used to derived mononuclear RNA)
4. Cell lysis buffer
5. RNA isolation kit
6. Substrates for real time RT-PCR (may vary for various real-time PCR techniques:
    poly dT primers, random hexamer primers
    Reverse Transcriptase and RT buffer
    DTT
    Deoxynucleotides 100 mM
    RNase H
    primer pairs for diagnostic and control gene set
    10x PCR reaction buffer
    Taq DNA polymerase
    Fluorescent probes for diagnostic and control gene set
    (alternatively, fluorescent dye that binds to only double stranded DNA)
    reaction tubes with or without barcode for sample tracking
    96-well plates with barcode for sample identification, one barcode for entire set, or individual barcode per reaction tube in plate
7. Software package for identification of diagnostic gene set from data
    Contains statistical methods.
    Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database server

8. Password and account number to access central database server.
9. Kit User Manual

FIGURE 4

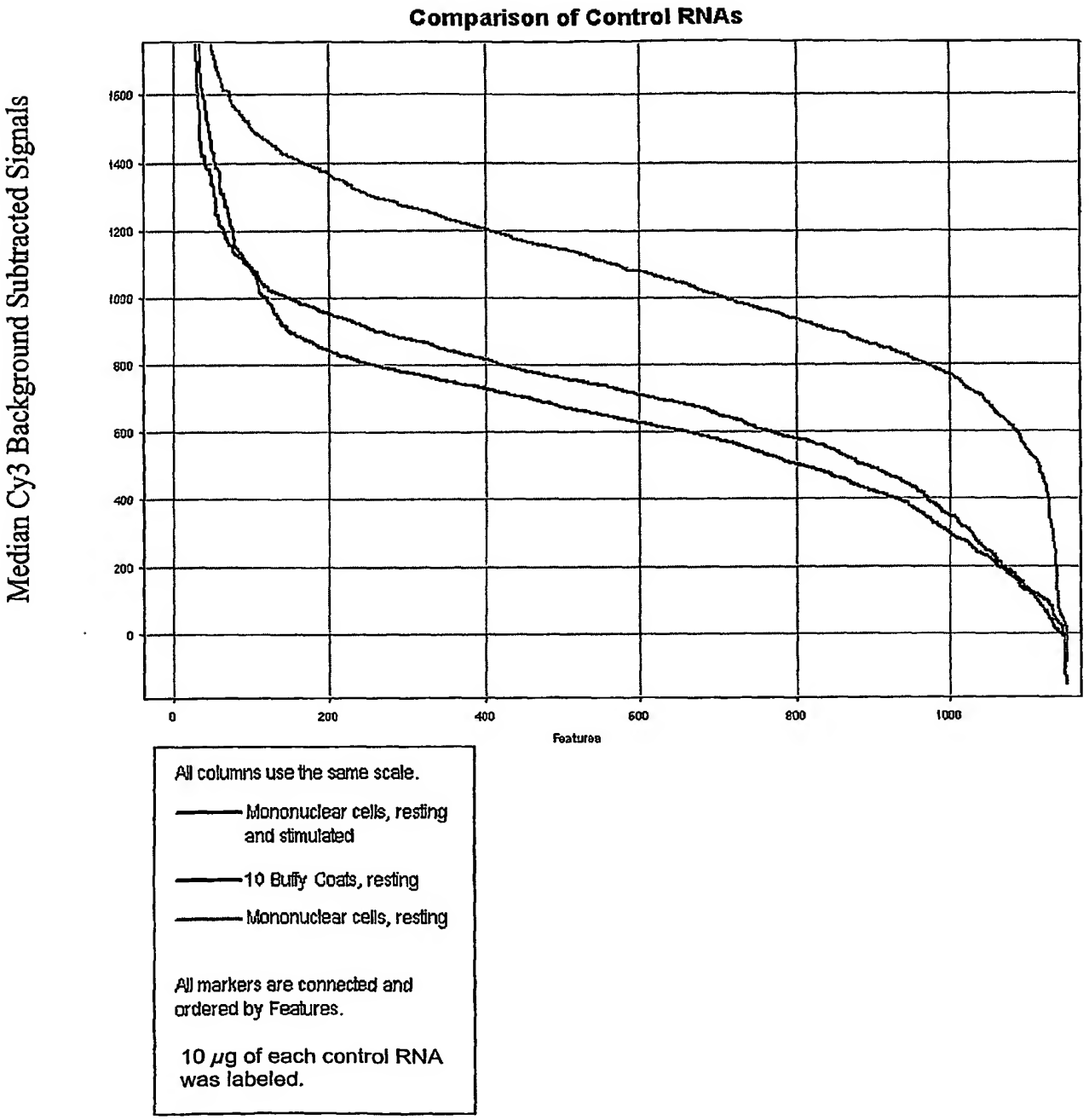
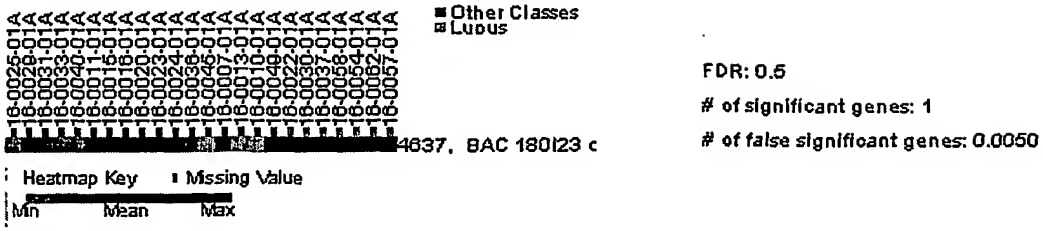

Comparison of Control RNAs
Median Cy3 Background Subtracted Signals
1600
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
Features
All columns use the same scale.
Mononuclear cells, resting and stimulated
10 Buffy Coats, resting
Mononuclear cells, resting
All markers are connected and ordered by Features.
10 μg of each control RNA was labeled.

Figure 5: SLE diagnostic genes and algorithms

A.



B.

Lupus		Control	
Sample	Ratio	Sample	Ratio
16-0022-01	1.05	16-0025-01	0.60
16-0030-01	0.96	16-0029-01	0.75
16-0037-01	0.87	16-0031-01	0.63
16-0058-01	1.05	16-0033-01	0.62
16-0054-01	0.99	16-0040-01	0.61
16-0062-01	0.98	16-0015-01	0.72
16-0057-01	1.14	16-0016-01	0.78
		16-0020-01	0.79
		16-0023-01	0.71
		16-0024-01	0.69
		16-0036-01	0.65
		16-0045-01	0.59
		16-0007-01	0.77
		16-0013-01	0.60
		16-0010-01	0.57
		16-0049-01	0.75

	Lupus	Controls
Average Ratio	1.00	0.68
Std Dev of Ratio	0.08	0.08
Fold Change	1.48	

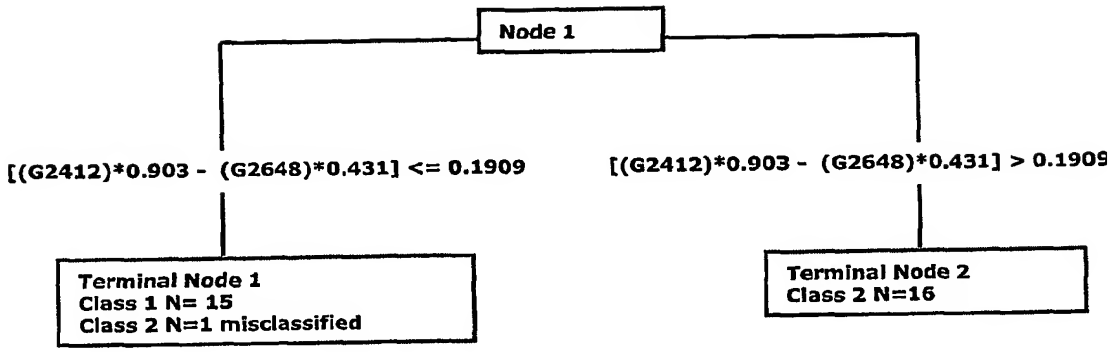
C.

Model	# genes	Relative Cost	SEQ ID 50mer	Locus	Nominal Description	CART Splitter	CART Value for Dx SLE
Model I	2	0.118	514	NM_002946	replication protein A2 (32kD)	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
			510	NM_004510	interferon-induced protein 75	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
Model I	3	0.125	514	NM_002946	replication protein A2 (32kD)	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
			510	NM_004510	interferon-induced protein 75	co-1st	[(2412)*0.903 - (2648)*0.431] <= 0.1909
			509	BC002409	actin, beta (ACTB)	2nd	(G1436) > 0.0868
Model II	1	0.612	504	W16552	PKR	1st	(5067) > 0.1030
Model II	3	0.686	504	W16552	PKR	1st	(5067) > 0.1030
			875	AK024756	hypothetical protein FLJ21103	2nd	(G1025) <= 0.3968
			876	AK024969	hypothetical protein DKFZp566I133	3rd	(G1035) <= 0.0073
Model II	5	0.745	504	W16552	PKR	1st	(5067) > 0.1030
			874	AK024240	cDNA FLJ14178 fis	2nd	(G1003) > 0.2105
			875	AK024756	hypothetical protein FLJ21103	2nd	(G1025) <= 0.3968
			873	AK024202	heat shock 90kD protein 1, alpha	3rd	(G1001) <= - 0.3107
			876	AK024969	hypothetical protein DKFZp566I133	3rd	(G1035) <= 0.0073

D.

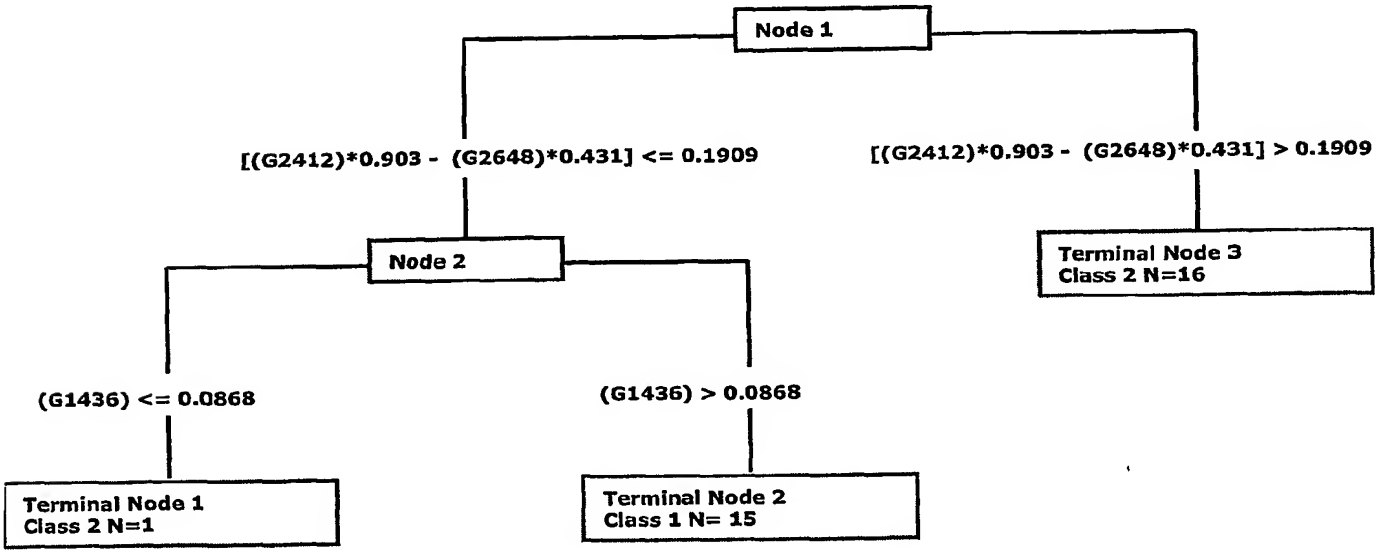
	Model	Sensitivity	Specificity	Relative Cost
Training Set	Model 1 (2 genes)	100	94	
	Model 1 (3 genes)	100	100	
10-fold Cross Validation	Model 1 (2 genes)	100	88	0.118
	Model 1 (3 genes)	93	94	0.125

E.



Model I (2 genes)

F.



Model 1 (3 genes)

Figure 6. Endpoint testing of PCR primers

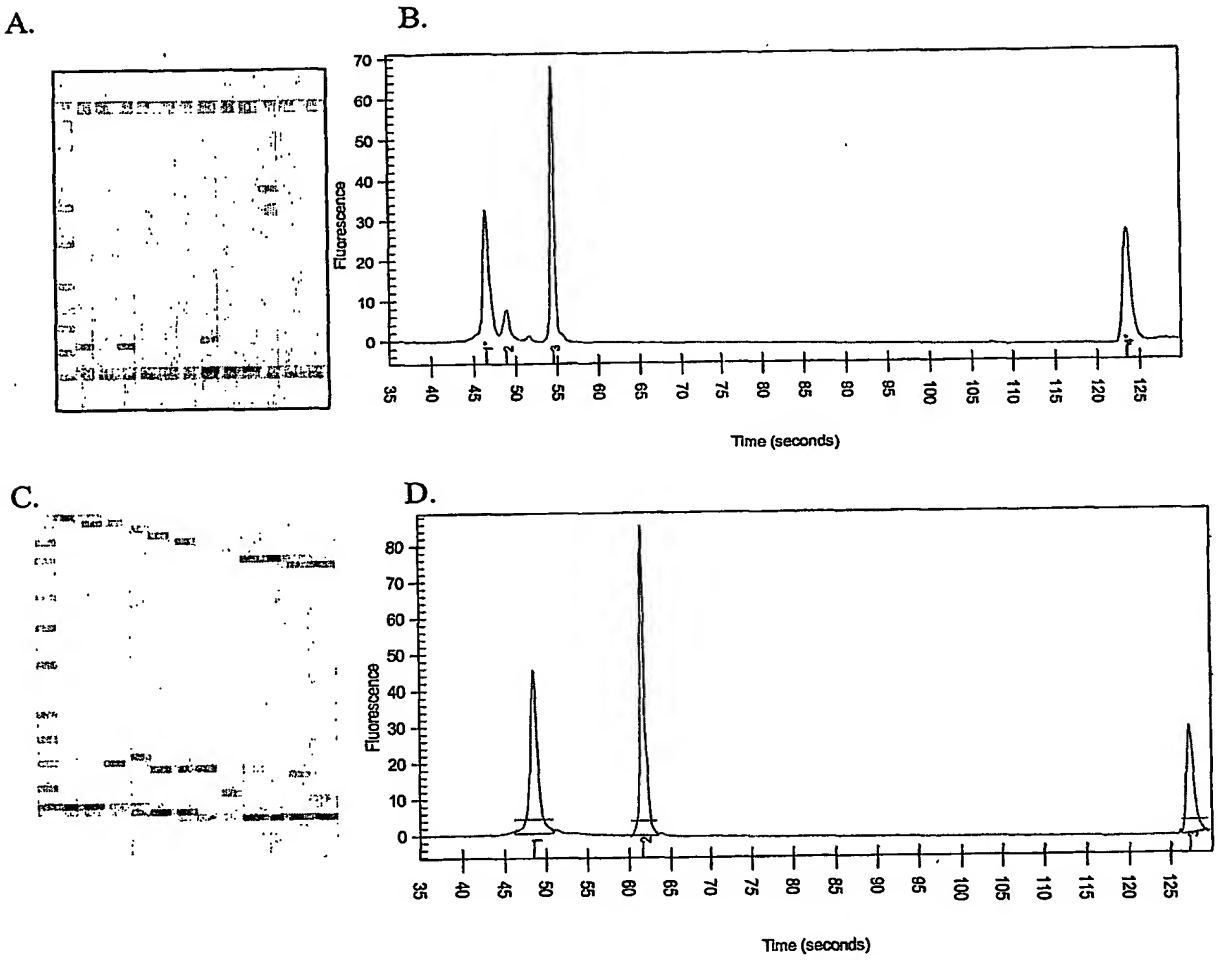
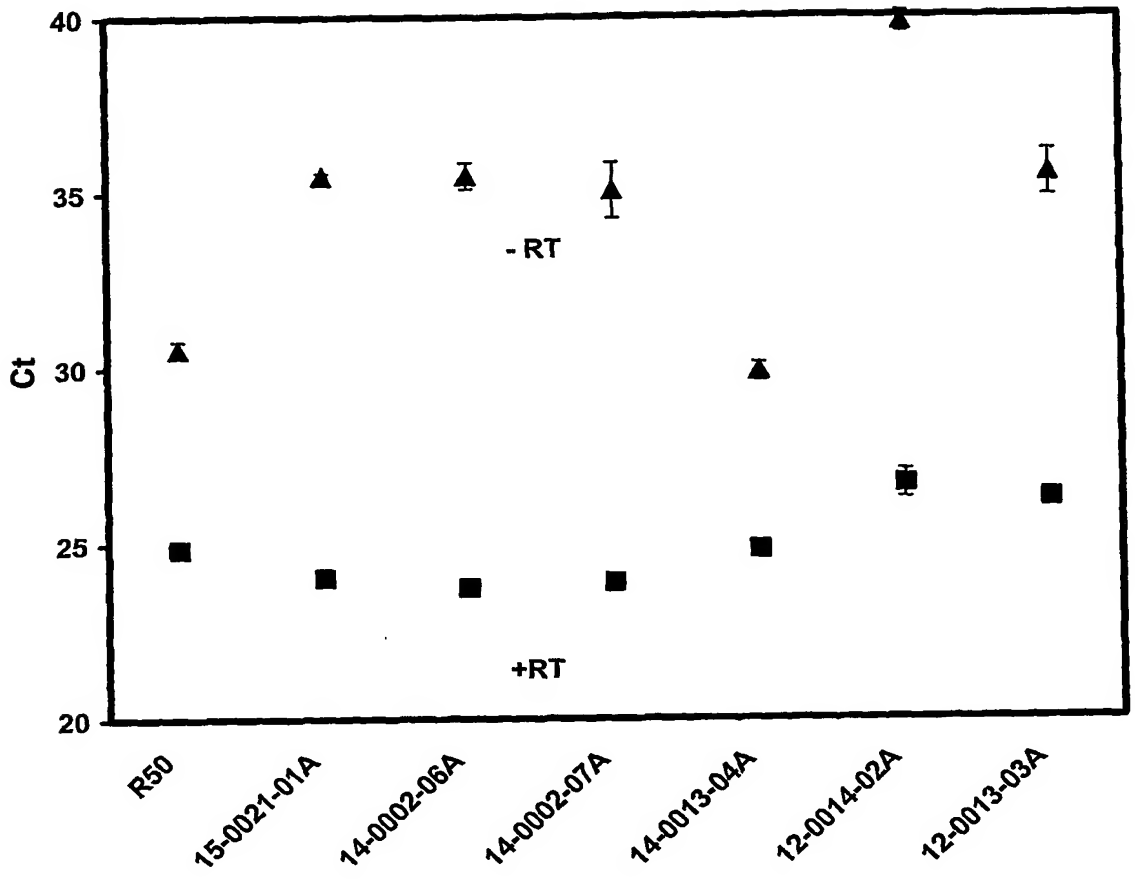


Figure 7: Validation of differential expression of Granzyme B in CMV patients using Real-time PCR

A.



B.

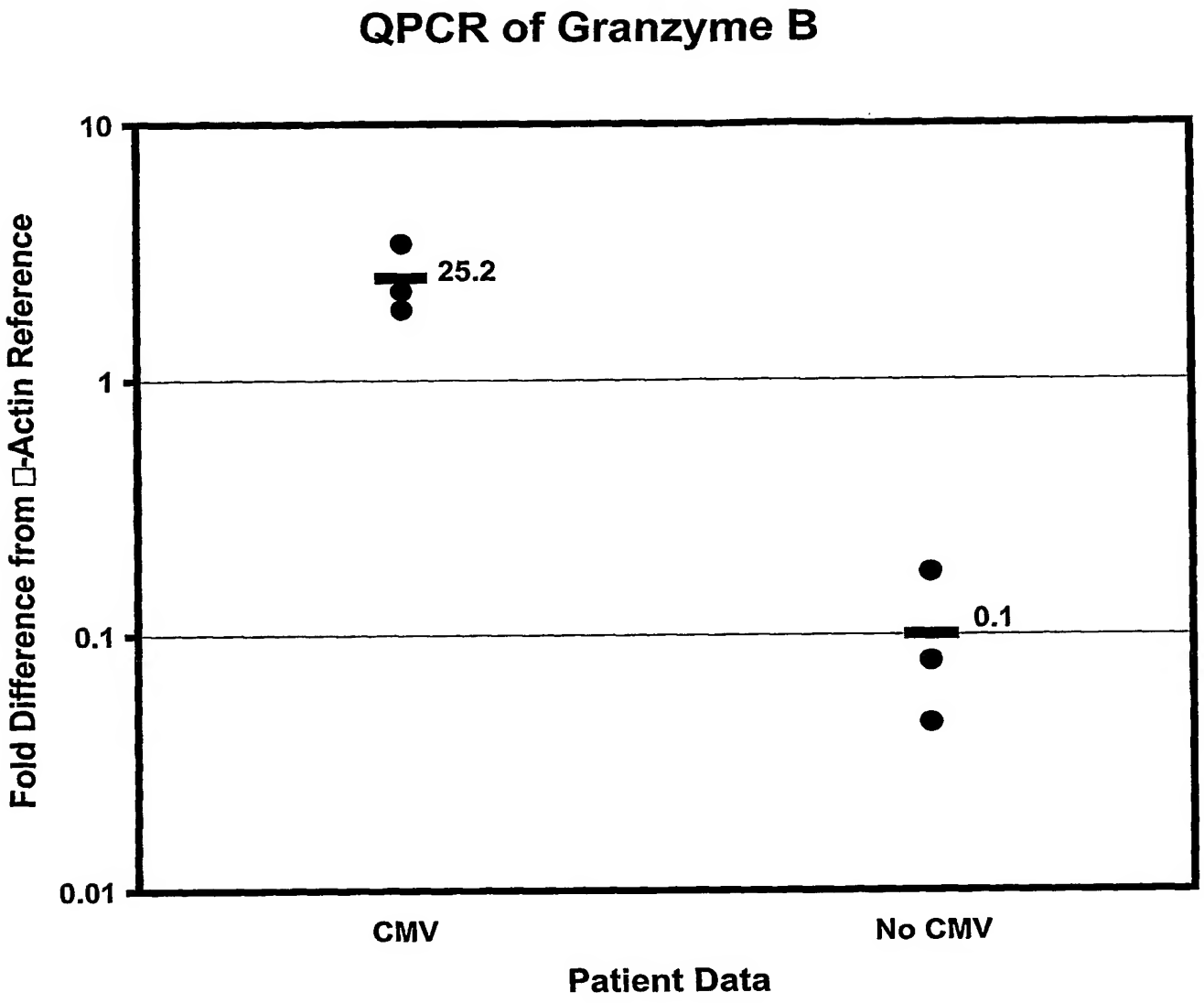
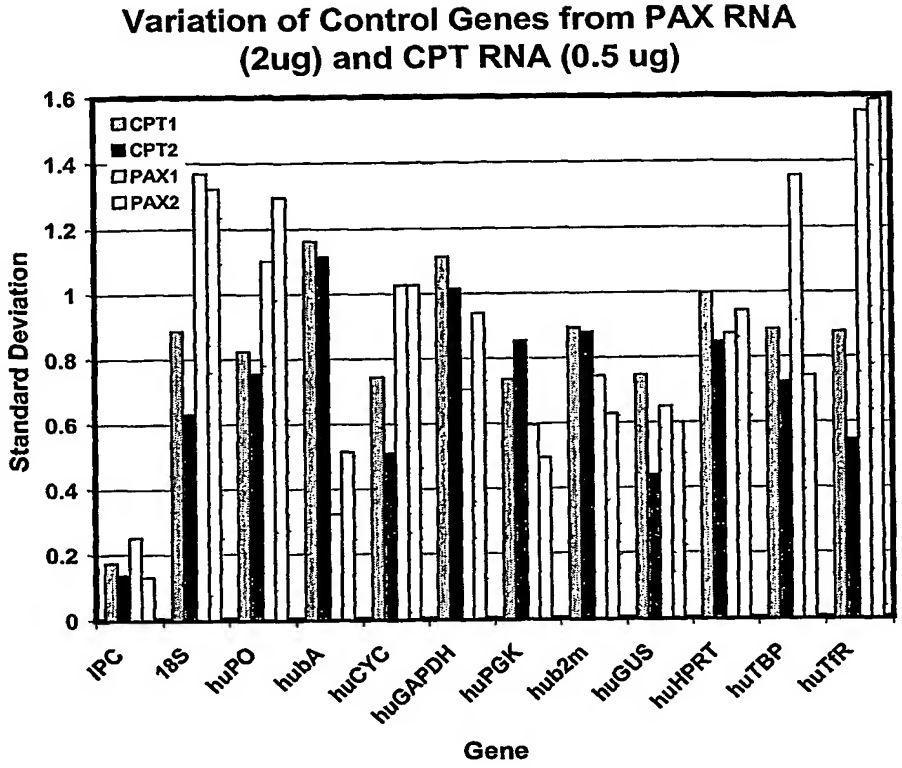
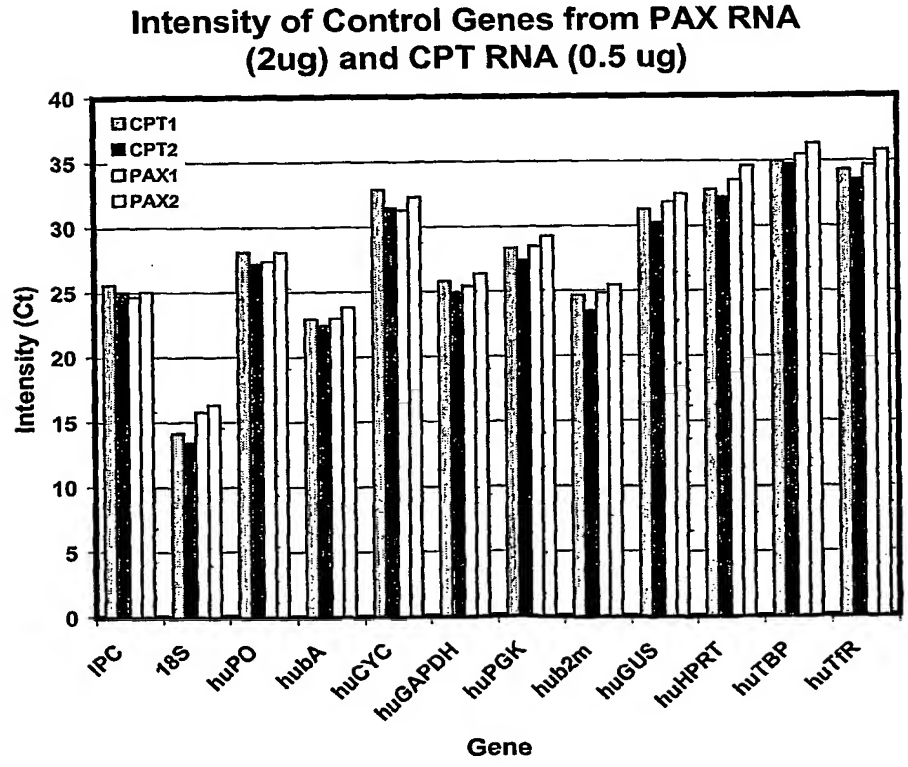

QPCR of Granzyme B
10
1
0.1
0.01
Fold Difference from □-Actin Reference
25.2
0.1
CMV
No CMV
Patient Data

Figure 8


Variation of Control Genes from PAX RNA (2ug) and CPT RNA (0.5 ug)
CPT1
CPT2
PAX1
PAX2
Standard Deviation
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IPC
18S
huPO
hubA
huCYC
huGAPDH
huPGK
hub2m
huGUS
huHPRT
huTBP
huTfR
Gene


Intensity of Control Genes from PAX RNA (2ug) and CPT RNA (0.5 ug)
CPT1
CPT2
PAX1
PAX2
Intensity (Ct)
40
35
30
25
20
15
10
5
0
IPC
18S
huPO
hubA
huCYC
huGAPDH
huPGK
hub2m
huGUS
huHPRT
huTBP
huTfR
Gene